Exploring the Effects of Collagen Glycation

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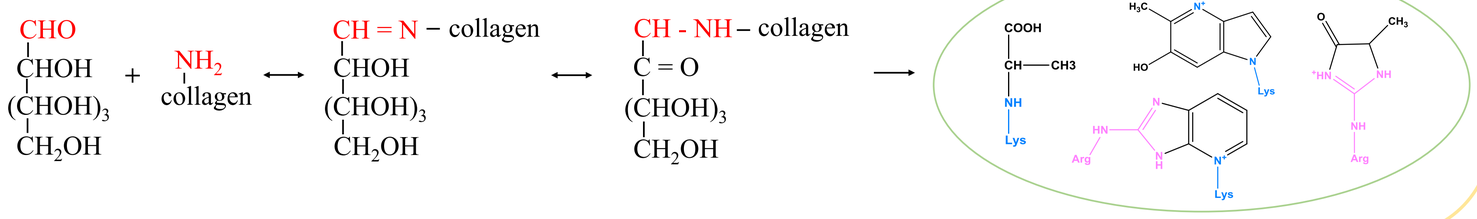
INTRODUCTION

- Diabetes and ageing both lead to similar symptoms, including multiple essential organ failure. Collagen glycation is widely believed to play an important role in this process.
- Extracellular matrix (ECM), a collagen assembled scaffold, as a long lived structural component with slow turn over rate is most likely to be affected by glycation, causing detrimental effects.
- Glycation is a non-enzymatic reaction between amino groups in proteins and aldehyde groups in sugars, causing amino acid sidechain modifications and forming intermolecular cross links. The reaction scheme and a few typical glycation products are shown below.



METHODS & OBJECTIVES

- Use mammalian cell culture method to produce Lys labelled ECM
- Incubate the ECM with reducing sugar
- Use multiple analytical methods, including solid-state NMR and SEM to examine the structural effects of glycation reactions on ECM
- Understand the chemistry behind ageing, diabetes and its complications on a molecular basis

RESULTS

Glycation Reaction between Extracellular Matrix and Ribose

Data included in this section are acquired from U-^{13}C, ^{15}N-Lys labelled ECM. Ribose involved in the experiment is also ^{13}C-enriched.

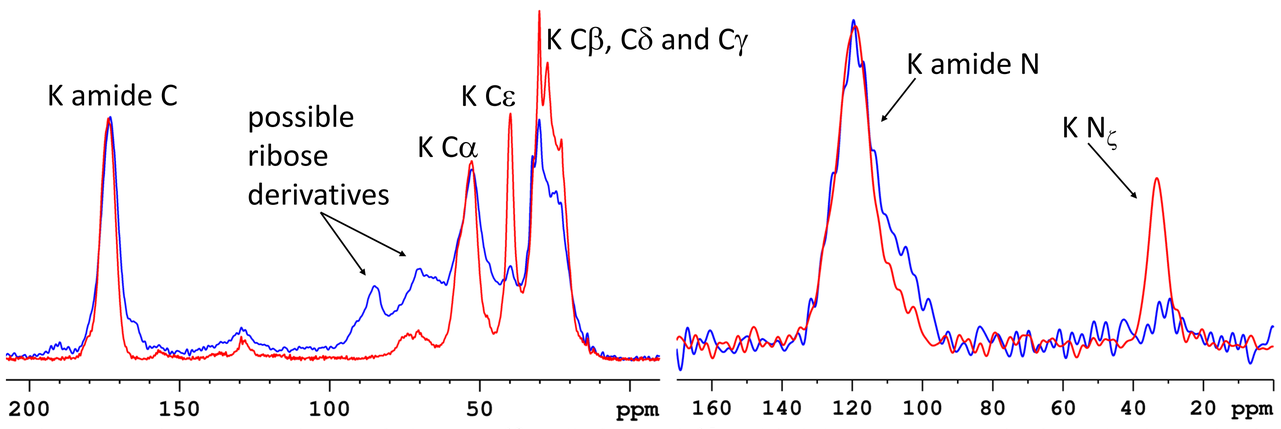


Figure 1 shows overlays of the 1D ^{13}C (left) and ^{15}N (right) CP spectra before (red) and after (blue) glycation reaction, confirming the occurrence of glycation reaction between collagen and ribose and the glycation site is Lys N_{ζ}.

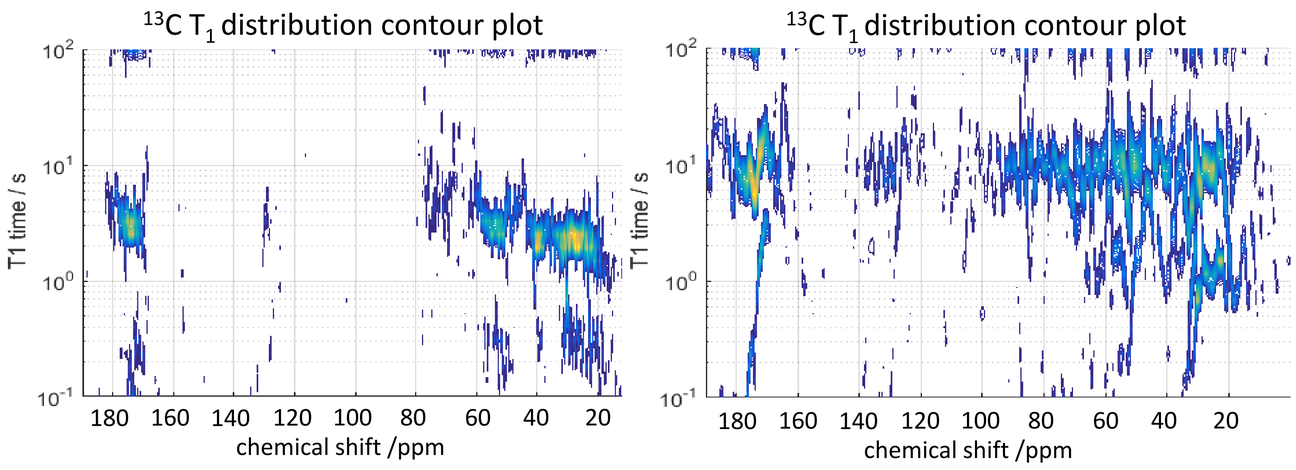


Figure 2 illustrates the dynamic changes before (left) and after (right) glycation reaction. Inverse Laplace Transform (ILT) separates the T_1 relaxation decay of different components and displays these as contour plots.

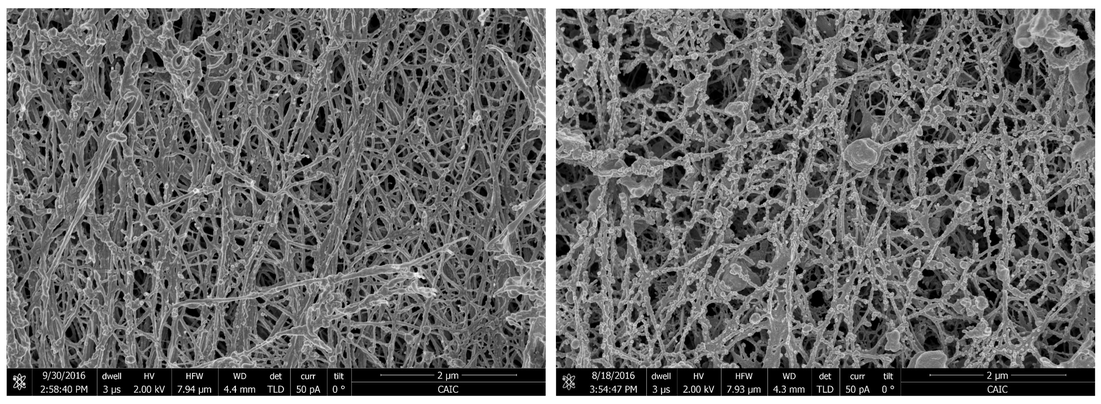


Figure 3 compares the SEM images before (left) and after (right) the glycation reaction.

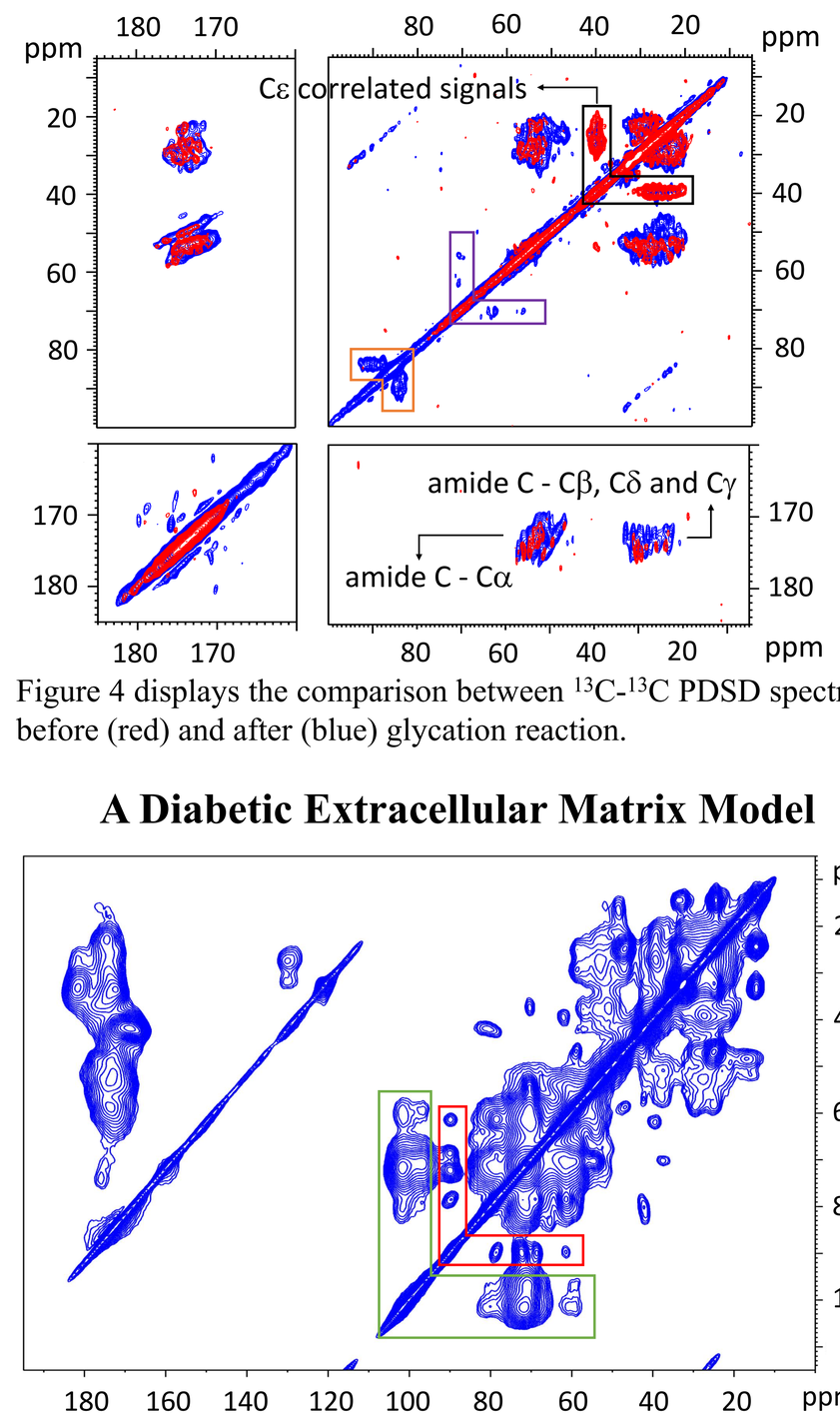
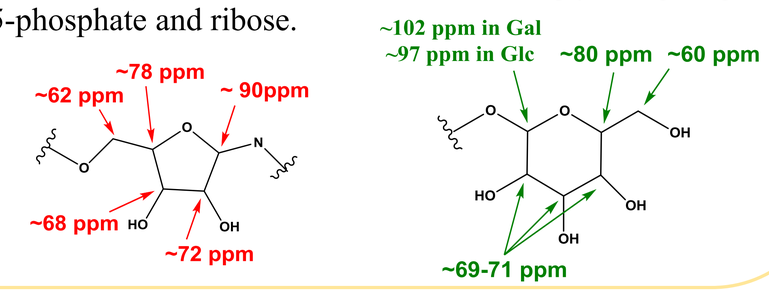


Figure 4 displays the comparison between ^{13}C-^{13}C PDSD spectra before (red) and after (blue) glycation reaction.

A Diabetic Extracellular Matrix Model

Figure 5 shows the ^{13}C-^{13}C DARR spectrum of a GlyLysGlc labelled ECM. Possible assignments are given below. This suggests that there are endogenous precursors of glycating sugars such as poly-(ADP-ribose) which can be converted to glycating sugars, ribose-5-phosphate and ribose.



FUTURE WORK

- Confirm as many assignments as possible based on current data
- Carry out 2D ^{13}C-^{15}N correlation experiments, helping assignments of more glycation products and ^{15}N relaxation experiments, studying the backbone dynamics change due to glycation
- Use paramagnetic probe to test the location of the modifications
- Modify the reaction condition and push the experiment towards the formation of advanced glycation end-products
- Link abnormal high sugar concentration to oxidative stress and examine the effects and products of oxidative stress

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